AD_____

Award Number:
W81XWH-11-1-0307

TITLE:
Biocompatible, Biodegradable, and Enzymatic-Cleavable MRI Contrast Agents for Early Detection of Bone Metastatic Breast cancer

PRINCIPAL INVESTIGATOR:
Xuli Wang, Ph.D.

CONTRACTING ORGANIZATION:

University of Utah
Salt Lake City, Utah 84112-8930

REPORT DATE:
April 2012

TYPE OF REPORT:
Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01/04/2012	Annual	04/01/2011 – 03/31/2012

4. TITLE AND SUBTITLE
Biocompatible, Biodegradable, and Enzymatic-Cleavable MRI Contrast Agents for Early Detection of Bone Metastatic Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0307

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Xuli Wang, Ph.D.
Email: xuliwang@utah.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Utah
Salt Lake City, Utah 84112

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland
21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This project proposes to design and test a novel peptide-based MRI contrast agent for early detection of bone metastasis from breast cancer. The proposed imaging agent is consist of bone targeting moiety of Asp8 and MRI imaging moiety of DOTA(Gd) with a cathepsin K cleavable peptide spacer. A solid phase peptide synthesis (SPPS) strategy was used to synthesize peptides. Coupling of the peptide onto DOTA followed by Gd complexation was performed to achieve the proposed imaging agent. Non-targeting and CTSK-insensitive controls were similarly prepared. The obtained contrast agents were characterized in terms of bone specificity, enzymatic degradability and biocompatibility. Preliminary testing of contrast enhanced MR imaging at different time points is ongoing in mice with bone metastasis from breast cancer.

15. SUBJECT TERMS
Breast cancer bone metastasis, Biomaterials, Peptide, MRI

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	UU	9	**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Introduction:

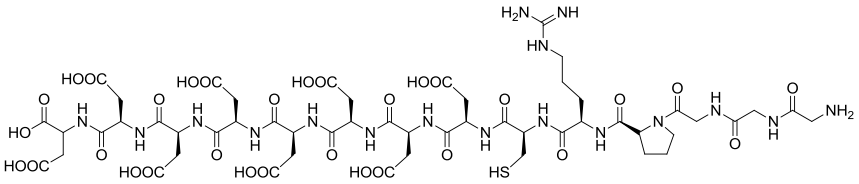
Metastatic breast cancers in bone are very difficult to treat and result in significant morbidity and mortality.(*1, 2*) Current therapeutic options for breast cancer induced bone-metastases are usually palliative, and planning an individual therapeutic strategy as early as possible to delay skeletal complications is the key to manage patients with bone metastases.(*3, 4*) Hence, more specific imaging technologies to early detect and accurately diagnose whether and what cancerous bone metastasis is greatly needed in clinical practice.(*3*) Currently skeletal scintigraphy represents the first line of diagnostic tools in the detection of osseous metastases.(*5*) However, it suffers from poor sensitivity to lytic bone tumors, which is the case for breast cancer metastases. It also cannot provide structural details of lesions in bone.(*5*) As a result, MRI is emerging as an alternative imaging modality for bone metastases diagnosis. Contrast enhanced MRI (CE-MRI) assisted with imaging probes can significantly improve early detection of tumor.(*6*) However, MRI used for the diagnosis of bone metastases in the current clinical setting, has not yet involved the use of contrast agents. Thus, the development of appropriate bone-specific MRI probes for bone metastases is of importance. This proposal addresses this important public health need.

The central hypothesis of this project is that Gd complex in conjugation with Lys-Nle-Pro-Gly-Gly-Asp8 will achieve a novel bone-targeted, biodegradable and enzymatic-cleavable MRI contrast agent. Lys provides an amine group to attach the Gd chelator. Asp8 has a high affinity for bone mineral and has been used as bone-targeting moiety in molecular therapeutics.(*7*) The use of Asp8 allows active accumulation of contrast agents in bone, thus leading to enhanced MR imaging in skeletal tissues. More importantly, by utilization of a CTSK-sensitive peptide linkage, Gd complex will be released following enzymatic cleavage. This not only reduces potential toxicity, but also leads to dynamic contrast enhancement at bone resorption sites to better detect CTSK activity. As CTSK is suggested as an indicator of bone metastasis from breast cancer,(*8*) probing CTSK activity at bone turnover sites may suggest the pathogenesis of bone metastasis. In summary, the research proposes a new bone specific contrast agent to explore molecular MR imaging for early detection of bone metastatic breast cancer.

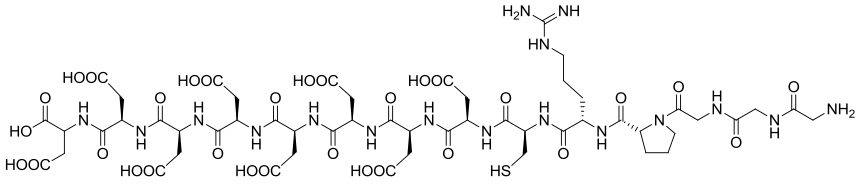
Body

Task 1 was to synthesize and purify peptide-based imaging agents. Proteolysis has been developed as a powerful tool for advanced drug delivery/imaging systems(*9, 10*). Cathepsin K (CTSK), a protease primarily resposible for bone resorption, has been identified as a valuable therapeutic target for osteoclast-mediated osteolytic disease.(*11*) Hence, CTSK-cleavable peptides have been expored in the development of molecular imaging probes and drug delivery systems in order to facilitate imaging agents/drugs release in osteolytic microenviroments. Peptides, e.g. RPGG, NPGG and HPGGPQ, have been demonstrated as CTSK-specific substrates in literature.(*12-15*) However, it is unclear which peptide sequence

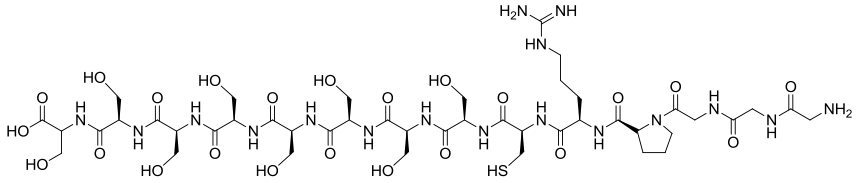
is more sensitive regarding to CTSK cleavage. To address this issue and identify best possible CTSK-cleavable linker for our project, we have synthesized CTSK-activatable imaging agents based on fluorescence resonance energy transfer (FRET) mechanisim. We found that upon CTSK treatment, fluorescence intensity increased up to 8.2, 2.5, and 7.6 fold in the case of using RPGG, NPGG and HPGGPQ as substrate, respectively. This study confirmed literatures' results that all of above mentioned peptides are more or less sensitive to CTSK treatment, making them suitable as CTSK-liable peptide for drug delivery or molecular imaging applications.(*12-15*) Orignially we proposed to use NPGG as CTSK substrate. Based on our preliminary results, however, we made appropriate adjustment to swith NPGG to RPGG as the alternative, and use HPGGPQ as the backup option. For this project, we have obtained 4 peptide which all were successfully synthesized by solid phase peptide synthesis (SPPS) strategy, and purified by preparative HPLC. The chemical structures of peptides were shown below. Peptides reacted with DOTA-NHS followed by complexing with Gd(OAc)3, and then afforded proposed peptide-based MRI contrast agents.



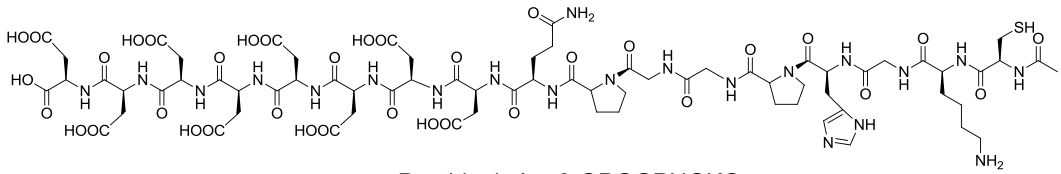
Peptide 1: $Asp_8CRPGGG\text{-}NH_2$



Peptide 2: $Asp_8C\text{-}rp\text{-}GGG\text{-}NH_2$



Peptide 3: $Ser_8CRPGGG\text{-}NH2$



Peptide 4: Asp8-QPGGPHGKC

Task 2 was to characterize peptide-based the contrast agents for the biophysical and biological properties. It was of improtance for such agents to possess skeletal binding

affinity, enzymatic degradability and biocompatibility. We confirmed that all of agents with Asp8 residue (bone binding moiety) indeed possess high HA binding affinity. In contrast, the control peptide with Ser8 residue only showed non-specific binding onto HA. In addition, we performed MTT assay in MDA-MB-231 (a breast cancer cell line). The resutls showed that non of agents indicated cytotoxicity to cells at concentration up to 1000 μg/mL, suggesting peptide-based imaging agents are safe and biocompatible as expected.

Task 3 is to investigate contrast enhanced MR imaging in mice. For this, the proposed imaging agent and also controls will be tested in mice with bone metastasis from breast cancer. The methods to establish animal model of bone metastasis from breast cancer are documented in literature.(*16*) (Rosol TJ, Tannehill-Gregg SH, LeRoy BE, Mandl S, Contag CH**. Animal models of bone metastasis**. *Cancer*, 97(3), 748-757 (2003)). We have submitted the IACUC protocol for this project, which was approved on December 15, 2010 and will expire until December 14, 2013. Right now we have difficulties to establish enough mice with breast cancer induced bone metastases to test our constructs. Non-cost extention of the research project (04/01/2012 t0 03/31/2013) was approved which allows us enough time to perform studies in Task 3.

Key Research Accomplishments

- 4 peptide-based imaging agents were successfully obtained.
- Preliminary test of imaging agents demonstrated that they are biodegradable, biocompatible and safe to use in animals.
- IACUC protocol was approved.

Reportable Outcomes

None at this time

Conclusion

This project proposes to design and test a novel peptide-based MRI contrast agent for early detection of bone metastasis from breast cancer. We have successfully obtained 4 peptides by using a solid phase peptide synthesis (SPPS) strategy. Coupling of the peptide onto DOTA followed by Gd complexation was performed to achieve the proposed imaging agents. Non-targeting and CTSK-insensitive controls were similarly prepared. The obtained contrast agents were characterized in terms of bone specificity, enzymatic degradability and biocompatibility. Preliminary testing of contrast enhanced MR imaging at different time points is ongoing in mice with bone metastasis from breast cancer.

References

1. Mundy, G. R. (2002) Metastasis to bone: Causes, consequences and therapeutic opportunities, *Nature Reviews Cancer 2*, 584-593.
2. Rose, A. A., and Siegel, P. M. (2010) Emerging therapeutic targets in breast cancer bone metastasis, *Future Oncol 6*, 55-74.
3. Walkington, L., and Coleman, R. E. (2011) Advances in management of bone disease in breast cancer, *Bone 48*, 80-87.
4. Verbeeck, A. (2004) Bone metastases from breast cancer: Guidelines for diagnosis, *Journal of Manipulative and Physiological Therapeutics 27*, 211-215.
5. Hamaoka, T., Madewell, J. E., Podoloff, D. A., Hortobagyi, G. N., and Ueno, N. T. (2004) Bone imaging in metastatic breast cancer, *Journal of Clinical Oncology 22*, 2942-2953.
6. Rodriguez, O., Fricke, S., Chien, C., Dettin, L., VanMeter, J., Shapiro, E., Dai, H. N., Casimiro, M., Ileva, L., Dagata, J., Johnson, M. D., Lisanti, M. P., Koretsky, A., and Albanese, C. (2006) Contrast-enhanced in vivo imaging of breast and prostate cancer cells by MRI, *Cell Cycle 5*, 113-119.
7. Wang, D., Miller, S. C., Kopeckova, P., and Kopecek, J. (2005) Bone-targeting macromolecular therapeutics, *Advanced Drug Delivery Reviews 57*, 1049-1076.
8. Le Gall, C., Bellahcene, A., Bonnelye, E., Gasser, J. A., Castronovo, V., Green, J., Zimmermann, J., and Clezardin, P. (2007) A cathepsin K inhibitor reduces breast cancer-induced osteolysis and skeletal tumor burden, *Cancer Research 67*, 9894-9902.
9. Law, B., and Tung, C. H. (2009) Proteolysis: A Biological Process Adapted in Drug Delivery, Therapy, and Imaging, *Bioconjugate Chemistry 20*, 1683-1695.
10. Woodward, J. K. L., Holen, I., Coleman, R. E., and Buttle, D. J. (2007) The roles of proteolytic enzymes in the development of tumour-induced bone disease in breast and prostate cancer, *Bone 41*, 912-927.
11. Zhao, Q., Jia, Y., and Xiao, Y. (2009) Cathepsin K: a therapeutic target for bone diseases, *Biochem Biophys Res Commun 380*, 721-723.
12. Lecaille, F., Choe, Y., Brandt, W., Li, Z., Craik, C. S., and Bromme, D. (2002) Selective inhibition of the collagenolytic activity of human cathepsin K by altering its S2 subsite specificity, *Biochemistry 41*, 8447-8454.
13. Lecaille, F., Weidauer, E., Juliano, M. A., Bromme, D., and Lalmanach, G. (2003) Probing cathepsin K activity with a selective substrate spanning its active site, *Biochem J 375*, 307-312.
14. Jaffer, F. A., Kim, D. E., Quinti, L., Tung, C. H., Aikawa, E., Pande, A. N., Kohler, R. H., Shi, G. P., Libby, P., and Weissleder, R. (2007) Optical visualization of cathepsin K activity in atherosclerosis with a novel, protease-activatable fluorescence sensor, *Circulation 115*, 2292-2298.
15. Kozloff, K. M., Quinti, L., Patntirapong, S., Hauschka, P. V., Tung, C. H., Weissleder, R., and Mahmood, U. (2009) Non-invasive optical detection of cathepsin K-mediated fluorescence reveals osteoclast activity in vitro and in vivo, *Bone 44*, 190-198.
16. Rosol, T. J., Tannehill-Gregg, S. H., LeRoy, B. E., Mandl, S., and Contag, C. H. (2003) Animal models of bone metastasis, *Cancer 97*, 748-757.

Appendices

IACUC approval Form

THE UNIVERSITY OF UTAH

AAALAC Accreditation (Health Sciences): 30 OCT 2008
AAALAC Accreditation (College of Science): 24 OCT 2008
PHS Assurance Registration Number: A3031-01
USDA Registration Number: 87-R-0001

Principal Investigator:	Xuli Wang
Protocol Number:	10-12004
Protocol Title:	Biocompatible, Biodegradable and Enzyme-Cleavable MRI Contrast Agents for Early Detection of Bone Metastatic Breast Cancer
Date of Approval:	15 DEC 2010
Date of Expiration:	14 DEC 2013

Protocol Summary:
This project has been approved for funding by the Dept. of Defense. Dr. Wang is the PI on this grant and is thus the PI on this IACUC application. However, Dr. Wang will not be directly involved with any animal procedures. This will all be done by the co-I, Dr. Miller and Ms. Mary Beth Bowman. Another co-I, Dr. Eun-Kee Jeong, will be responsible for operation of the MRI, but not directly involved with animal procedures. This project proposes to develop a novel bone-specific MRI contrast agent that should improve the early detection of breast cancer metastases to skeletal tissues. The agent consists of a bone-seeking moiety and with a bone-specific enzyme cleavable linkage to improve biocompatibility and reduce toxicity. The enzyme cleavable linkage will also permit the detection of local enzyme activity that can be used as an indication of disease activity and progression. The new construct will be tested in mouse metastatic breast cancer model.

Your animal protocol was reviewed at a convened IACUC meeting and approved on the date listed above.

Please be aware that serious or repeated adverse events (e.g., a large number of postoperative complications, excessive or unexpected mortality rate) must be reported timely to the IACUC committee. The notification should include a brief summary of the adverse event and any corrective actions. It is further required to report if any of the adverse events lead to a change in pain categories (e.g., unalleviated pain or severe distress, category E).

For your convenience a copy of your approved protocol, that includes supportive documents, is enclosed.

_____ IACUC

12/15/10 Date

cc: Thomas N. Parks, Ph.D.
Vice President for Research

IACUC
Phone: (801) 581-5950
Email: iacuc@ocm.utah.edu